UTILIZATION OF PHENOLS AND BIOSYNTHESIS OF HUMIC ACID BY *ASPERGILLUS TERREUS*

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ABSTRACT. - In cultures supplemented with catechol, p-hydroxybenzoic acid, hydroquinone, resorcinol and salicylic acid as sole C sources, *Aspergillus terreus* grew successfully but growth rate varied. However, the fungus failed to grow on phloroglucinol even at 0.05% level. Increasing concentration of phenols from 0.05 to 0.15% was accompanied by increase in growth rate in case of resorcinol and salicylic acid whereas growth was suppressed with higher concentrations of other compounds tested. Humic acid synthesis was also inhibited by increasing concentrations of catechol, *p*-hydroxybenzoic acid and resorcinol. When culture media were ammended with 0.5 glucose/l, growth inhibition was alleviated, phenols utilization accelerated, inhibition in humic acids formation disappeared and fungicidal action of phloroglucinol delayed.

RÉSUMÉ. - *Aspergillus terreus* présente un développement variable sur les milieux de culture contenant du catechol, de l'acide p-hydroxybenzoïque, de l'hydroquinone, du resorcinol ou de l'acide salicylique comme seule source de carbone. Au contraire, aucune croissance n'est observée en présence de phloroglucinol, même à la concentration de 0.05%. Des concentrations croissantes de phénols de 0.05 à 0,15% stimulent la croissance dans le cas du résorcinol et de l'acide salicylique. Elle est au contraire inhibée par des concentrations croissantes des autres composés. La synthèse d'acides humiques est également inhibée par des concentrations croissantes de catechol, d'acide p-hydroxybenzoïque ou de resorcinol. Quand le milieu de culture est enrichi en glucose (0.5g/l), l'inhibition de croissance est évitée, la consommation de phénols augmente, l'inhibition de la synthèse d'acides humiques disparaît et l'action fungicide du phloroglucinol n'est plus observée.

KEY WORDS. - *Aspergillus terreus*, humic acid, phenols.

INTRODUCTION

Natural habitats continuously receive phenols either directly or from the degradation of the lignin component of plant wastes and transformation of aromatic compounds (Brown, 1969; Stanier & Ornston, 1973; Chen & Chen, 1985; Hartley & Whitehead, 1985).

Phenolic monomers when released in soil affect the growth of microorganisms, major agents of mineralization and utilization of organic pollutants in terrestrial and aquatic environments. Microbial enzymatic activities involved in the utilization of soil organic nutrients helps in the removal of wastes (Alexander, 1981). Martin & Akin (1988) found that phenolic monomers inhibited the enzymatic activities of *Bacteroides rumicola* and *B. succinogenes* involved in the utilization of polysaccharidic components of lignocellulose. Search of phenol-utilizing microorganisms, is thus of a particular concern. Utilization of organic chemicals by soil microbes depend upon the type of chemical, concentration and presence of organic matter (Stevenson, 1972). Haider & Martin (1967) reported that phenols could be polymerized by soil microbes to humic

substances. In addition, the ability of soil microbes especially cellulose decomposers to utilize phenols is of ■ great importance in straw decomposition and disposal of plant wastes. This is because straw contains about 1-3% phenolic compounds that restrict its degradation (Hartley & Whitehead, 1985). Utilization by fungi of phenols as sole carbon sources were discussed in earlier investigations (Turner & Rice, 1975; Black & Dix, 1976).

The fungus *Aspergillus terreus* is a major inhabitant of straw and is involved in straw breakdown. The present study was made to evaluate its ability to develop on a range of phenolic compounds, used as sole carbon sources or in presence of glucose, and to synthesize humic acid.

MATERIALS AND METHODS

An isolate of *Aspergillus terreus* Thom recovered from wheat straw as a cellulose degrading fungus (Eggins & Pugh, 1962) was used for this investigation. The organism was maintained on malt extract agar at 30°C before use.

Growth conditions

The fungus was grown in 250 ml conical flasks with 50 ml of glucose-free Czapek's liquid medium supplemented with membrane sterilized phenol solution to give concentrations of 0.05, 0,1 and 0.15% (W/V). The following phenolic compounds were used: *o*-dihydroxybenzene (catechol), *p*-hydroxybenzoic acid, *p*-dihydroxybenzene (hydroquinone), *m*-dihydroxybenzene (resorcinol), phloroglucinol and *o*-hydroxybenzoic acid (salicylic acid). Three flasks were used for measuring CO_2 evolution, utilization of phenols and estimation of mycelial growth. Three other flasks were employed for evaluation of humic acid synthesis. Same levels of phenols were also used in Czapek's medium ammended with 0.5 g glucose/l.

Measuring of growth, CO_2 evolution and phenol utilization

For these purposes, 20 ml of 0.5 N NaOH in a glass vial were introduced in each flasks to trap CO_2. Flasks were fitted with rubber stoppers with inlet and outlet side arms. Arms were closed and flasks were incubated at 30°C for 15 days as static cultures. To provide aeration, flasks were flushed periodically with CO_2-free air to prevent growth inhibition. At the end of the experiment, CO_2-C in traps was estimated by the conductivity method (Wollum & Gomez, 1970). Flasks contents were then filtered through preweighed filter paper. Mycelia collected were rinsed twice with distilled water to remove adsorbed phenols, dried and weighed. Filtrates were used for estimation of residual phenols after precipitation of protein using Folin reagent (Swain & Hillis, 1959; Amorim et al., 1977). Phenol consumed was calculated by deducing residual phenol from that originally used.

Estimation of humic acid C

Flasks used for estimation of humic acid C were incubated under the same conditions as above except that no NaOH was used. At the end of the incubation period, flasks contents were dried out and extra- and intracellular humic acids C was estimated by the procedure adopted by Malik et al. (1979) and Jain et al. (1979). Dried material of each flask was treated with 20 ml of 0.5 N NaOH per g of dried materials, kept

overnight and centrifuged to separate the supernatant. The supernatant was made up to 50 ml in a volumetric flasks. For determination of humic acid C, 5 ml portion was acidified with 0.5 NH_2SO^4 to pH 1.0, kept at 90°C for 30 min, left overnight. Then centrifuged to separate the dark-coloured precipitate of humic acid. The precipitate was redisolved in 0.1 N NaOH to make the volume to 50 ml and a 20 ml sample was taken for C estimation. Carbon was estimated by adding 8 ml H_2SO_4 and 2 ml 2N $K_2Cr_2O_7$ to 20 ml of humic acid extract, keeping the reaction mixture in ice bath. The mixture was kept at 110°C for 1.5 h along with a blank. Absorbance was noted at 590 nm and C in samples was estimated using glucose-C standard.

RESULTS AND DISCUSSION

The effect of phenolic compounds on growth of *A. terreus* (Table 1) indicate that growth was successful on all phenolic used as sole C source at 0.05% level. Higher concentrations of phenols up to 0.15% also increased the growth rate in case of resorcinol and salicylic acid but the reverse was observed with other compounds tested.

Table 1. - Growth, respiration, phenol utilization and synthesis of humic acid by *A. terreus* in cultures amended with referent phenolic compounds as sole C-sources.

Tableau 1. - Croissance, respiration, consommation des phénols et synthèse d'acides humiques par *A. terreus* cultivé sur des milieux ne contenant que des composés phénolés comme seule source de carbone.

Compounds	Concentration %	Dry weight (mg/100 ml)	CO^2C collected (mg)	Phenol utilized (% of applied)	Humic C (mg/100 ml)
Catechol	0.05	91.7	3.2	84.1	10.8
	0.10	87.5	3.7	82.2	9.5
	0.15	83.4	4.7	72.5	9.0
p.hydroxy-benzoic acid	0.05	106.2	5.7	84.8	10.6
	0.10	84.4	5.9	82.1	9.8
	0.15	76.2	6.6	78.0	8.5
Hydroquinone	0.05	89.8	3.1	80.0	9.3
	0.10	80.2	3.3	79.0	9.8
	0.15	75.0	2.4	78.0	13.3
Resorcinol	0.05	64.0	3.2	72.6	10.9
	0.10	95.8	4.4	83.1	9.8
	0.15	120.0	6.8	84.0	9.7
Salicilic acid	0.05	82.6	5.7	80.0	9.8
	0.10	96.6	5.4	86.0	9.9
	0.15	103.0	5.6	90.6	10.4

Each value represents the average of three replicates.

A. terreus failed to grow in cultures containing phloroglucinol as C source (result not presented). CO_2-C evolution was promoted by the increase in phenol concentration in cultures with catechol, p-hydroxybenzoic acid and resorcinol but not with other phenols. However, some of CO_2-C may be arose from endogenous metabolism of inoculum. Humic acid C formation was recorded in all cases and phenol utilization, ex-

cept of catechol and p-hydroxybenzoic acid, either was increased or not influenced by increasing concentrations. Increase in phenol utilization, except of catechol and p-hydroxybenzoic acid, either was increased or not influenced. Increase in phenol utilization was accompanied by an increase in CO_2 evolution or biomass formation and/or humic acid synthesis. In this respect, anthracene, naphthalene and other aromatic hydrocarbons were metabolized by several fungi (Cerniglia et al., 1978; Barkay & Pritchard, 1988) but reports about phenols utilization and degradation are still few. Regarding results in Table 1, *A. terreus* grown on media with phenols as sole C source, synthesize humic acid and liberate CO_2. Utilization of these substances as C sources may undergo different pathways. One of the presumed ways is the degradation of these compounds via removal of side-chains groups and ring opening to produce simple organic molecules and release CO_2 (Alexander, 1983). Another pathway is the polymerization to humid substances (Haider & Martin, 1967). Utilization of phenolic acids as sources was earlier reported (Turner & Rice, 1975; Black & Dix, 1976). In agreement with our results, humic acid C production by yeast was linearly related to concentration of hydroquinone (Hasset et al., 1988). Schnitzer et al. (1984) referred the effect of phenol on humic acid formation to the effect of phenol concentration on the enzymatic polymerization (humification) of phenol.

When the growth media was supplemented with glucose (Tab. 2) mycelial growth patterns of *A. terreus* in presence of phenols varied. Added glucose alleviated growth inhibition of catechol and *p*-hydroxybenzoic, and markedly promoted growth in presence of salicylic acid. Also, addition of glucose to phloroglucinol made growth of *A. terreus* possible. Phenol utilization also increased with glucose. Inhibition of humic acid formation by some phenols was alleviated by glucose addition In media supplemented with other carbon sources, *Epicoccum nigrum* transformed phenol to humic acid (Haider & Martin, 1967). In another investigation, Martin & Haider (1979) studied the effect of readily available carbon substrate on the biodegradation of phenolic substances in various soils; addition of 0.5% orange leaves with phenols exerted no measurable effect on the rate of degradation. From Tables 1 and 2, addition of glucose increase phenol utilization and humic acid synthesis, probably due to rapid establishment of growth in presence of glucose. Also, inhibition of humic acid formation by some phenols was alleviated by glucose addition. This effect may be due to activation in the production of phenol polymerizing enzymes that may have decreased at higher phenol concentrations. A similar observation was noted by Blum & Shafer (1988); phenolic acid was readily utilized by microorganisms in soil when adequate nutrients were present.

In conclusion, *A. terreus* grows successfully in cultures having phenols as sole C sources but some phenols at higher concentration decreased growth and humic acid formation. This inhibition was alleviated with the addition of glucose at the rate of 0.5 g/L. This observation could be of certain value in the disposal of such toxic and pollutant chemicals. Also, the capability of microorganisms, especially cellulose decomposers as *A. terreus,* to utilize or degrade phenols accelerate disposal of plant wastes whose degradadation is restricted by the phenolic compounds of their lignin component as in stubble (Hartley & Dahona, 1981). Other advantages of this work include synthesis of humic substances that increase soil fertility.

Table 2. - Growth, respiration, phenol utilization and synthesis of humic acid by A. terreus in cultures amended with phenols and 0.5 g glucose/L as C-sources.

Tableau 2. - Croissance, respiration, consommation des phénols et synthèse d'acides humiques par A. terreus cultivé sur des milieux contenant des composés phénolés et 0.5 g/l de glucose comme source de carbone.

Compounds	Concentration %	Dry weight (mg/100 ml)	CO^2C collected (mg)	Phenol utilized (% of applied)	Humic C (mg/100 ml)
Catechol	0.05	104.0	5.4	90.3	11.5
	0.10	107.3	5.9	92.2	11.2
	0.15	107.6	8.7	88.5	11.3
p.hydroxy-benzoic acid	0.05	118.0	6.9	86.4	11.2
	0.10	125.0	6.5	88.6	11.4
	0.15	122.0	8.3	85.3	10.9
Hydroquinone	0.05	94.9	4.1	83.6	9.5
	0.10	106.6	5.2	82.2	10.3
	0.15	135.4	6.0	84.1	13.5
Phloroglucinol	0.05	67.4	3.9	30.8	5.6
	0.10	57.8	3.6	28.1	5.2
	0.15	80.3	2.7	35.0	6.2
Resorcinol	0.05	120.0	6.1	85.4	11.5
	0.10	132.8	6.8	88.6	11.1
	0.15	140.4	8.4	93.5	11.2
Salicilic acid	0.05	103.4	5.8	83.3	10.0
	0.10	132.0	7.2	88.5	10.9
	0.15	147.2	10.8	94.2	10.8

Each value represents the average of three replicates.

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